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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Heiko RAUER, et al. GAU: 1614

SERIAL NO: 10/376,264 EXAMINER:

FILED: March 3, 2003

FOR: NOVEL MODULATORS OF POTASSIUM CHANNELS

REQUEST FOR PRIORITY

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

☐ Full benefit of the filing date of U.S. Application Serial Number , filed , is claimed pursuant to the provisions of **35 U.S.C. §120**.

■ Full benefit of the filing date(s) of U.S. Provisional Application(s) is claimed pursuant to the provisions of **35 U.S.C. §119(e)**:

Application No.	**Date Filed**
60/361,299	March 4, 2002

☐ Applicants claim any right to priority from any earlier filed applications to which they may be entitled pursuant to the provisions of **35 U.S.C. §119**, as noted below.

In the matter of the above-identified application for patent, notice is hereby given that the applicants claim as priority:

COUNTRY	**APPLICATION NUMBER**	**MONTH/DAY/YEAR**
GERMANY	102 09 520.5	March 4, 2002

Certified copies of the corresponding Convention Application(s)

- ■ are submitted herewith
- ☐ will be submitted prior to payment of the Final Fee
- ☐ were filed in prior application Serial No. filed
- ☐ were submitted to the International Bureau in PCT Application Number
  Receipt of the certified copies by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.
- ☐ (A) Application Serial No.(s) were filed in prior application Serial No. filed ; and
- ☐ (B) Application Serial No.(s)
  - ☐ are submitted herewith
  - ☐ will be submitted prior to payment of the Final Fee

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Prioritätsbescheinigung über die Einreichung einer Patentanmeldung

Aktenzeichen:	102 09 520.5
Anmeldetag:	4. März 2002
Anmelder/Inhaber:	4SC AG, Planegg/DE
Bezeichnung:	Novel modulators of potassium channels
IPC:	A 61 K 31/403

Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der ursprünglichen Unterlagen dieser Patentanmeldung.

München, den 1. April 2003
Deutsches Patent- und Markenamt
Der Präsident
Im Auftrag

Faust

A 9161
03/00
EDV-L

NOVEL MODULATORS OF POTASSIUM CHANNELS

The present invention relates to potassium channel modulating indole derivatives. These compounds are useful in the treatment or alleviation of disorders and conditions associated with, or dependent on the membrane potential or conductance of cells in mammals, including a human. The present method also provides a method for the manufacture of medicaments and pharmaceutical compositions comprising the K^+ channel modulating agents. The agents of the invention are useful for the treatment or alleviation of diseases, disorders, and conditions associated with or responsive to the modulation of potassium channels.

Potassium channels (K^+ channels) are present in nearly all cells and play a crucial role in a wide variety of cellular regulation processes due to modulation of the membrane potential. K^+ channels can be regulated by changes in membrane voltage, internal Ca^{2+} concentration, phosphorylation, and multiple other cellular mechanisms (Hille, B., Ionic channels in excitable membranes, 2nd ed., *Sinauer Assc.* (1992)). The family of potassium channels can be divided into several subfamilies, one being the group of Ca^{2+}-activated K^+ channels. The potassium channel BK belongs to this subfamily of Ca^{2+}- activated K^+ channels (K_{Ca}) and shows a large single channel conductance of ~150pS. The BK channel (or MaxiK), encoded by the *Slo* gene, is mainly regulated by the internal Ca^{2+} concentration and membrane voltage as well as ß-subunit modulation, phosphorylation states, and other cellular mechanisms (Nelson M.T. et al., *Science* 270, 633-637 (1995); Levitan, I.B., *Annu. Rev. Physiol.*, 56, 193-212 (1994) ; Vergara *et al.*, *Curr. Opin. Neurobiol.*, 8, 321-329 (1998); McManus, O.B., *Neuron*, 14, 645-650 (1995)). Large conductance, Ca^{2+}-activated BK channels are ubiquitously expressed, except in myocardial tissue, and play a key role, e.g. in smooth muscle tone, neuron firing, and cell secretion (Toro, L. *et al.*, From ion channels to cell to cell conversations, *Plenum Press*, NY 47-65, (1997); Fox, A.J. *et al.*, *J. Clin. Invest.*, 99, 513-519 (1997); Nelson M.T. *et al.*, *Science* 270, 633-637 (1995); Lingle C:J. *et al*, *Ion channels*, 4, 4, 261-301 (1996)). The opening of BK channels leads to a shift of the membrane potential towards the potassium reversal potential causing hyperpolarization of the cell. Due to its large single channel conductance the opening of only few BK channels can produce a significant leftward shift of the

membrane potential due to the increased K^+ conductance. Such mechanisms are important for example in smooth muscle cells, where hyperpolarization caused by BK channel opening leads to a relaxation and therefore a reduced vascular tone, or in neuronal tissue, where BK channel opening counteracts depolarisation and can limit the hyperactivating and/or damaging Ca^{2+} entry under different disease conditions. Inhibition of BK channels can maintain or lead to a more depolarized membrane potential of the cell and therefore maintain or prolong cellular processes depending on cellular depolarization.

Other members of the subfamily of Ca^{2+}-activated K^+ channels (K_{Ca}) are SK_{Ca} (SK_{Ca}-1,2,3) and IK_{Ca} channels, with small or intermediate conductances, respectively. SK_{Ca} and IK_{Ca} channels do not show any voltage dependence like the BK channel described above. SK_{Ca} channels are expressed in different neuronal tissues, in skeletal muscles, gland cells, liver cells, lymphocytes, and other peripheral cells. SK_{Ca} channels are important in mechanisms, where a specific regulation of the cellular membrane potential is required for the normal function of cells, e.g. the after-hyperpolarization in neuronal tissues influencing the firing pattern of neurons. IK_{Ca} channels are expressed, e.g. in endothel cell, red blood cells, and lymphocytes. These channels are also responsible for a tightly regulated membrane potential to guarantee a specific cellular function, e.g. the activation processes of T-lymphocytes. Other K^+ channels that are important for a specific regulation of the membrane potential are K_{ATP} channels. These K^+ channels belong to the subfamily of channels with 2 transmembranal segments and are inhibited by intracellular ATP. These channels are expressed, e.g. in insulin secreting cells or in vascular muscles, where they have an important role in regulating vascular tone (for review see Coghlan *et al., J. Med. Chem.*, 44, 1627-1653 (2001).

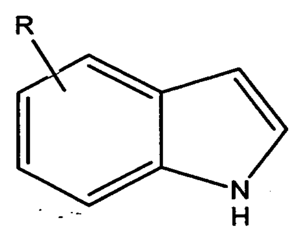
In general, modulation of K^+ channels by agonistic or antagonistic compounds can influence the membrane potential of K^+-expressing cells, enabling a specific modulation of cells and/or tissues that might be useful in the treatment of diseases linked to membrane potential or conductance dependent cellular functions.

Several natural and synthetic molecules with the ability to modulate K^+ channels have been identified in the past. Examples of such compounds are the avena pyrone with BK channel opening activity (WO 93/08800), triaminobenzene analogues were reported to show K^+ channel opening activity (US 5,200,422), the aryl-pyrrole NS-8 has been disclosed to act as

a K^+ channel opener useful in the treatment of bladder dysfunction (Tanaka, et al., *J.Urol.* 159, 21 (1998)), indole-3-carboxylic acid esters have been shown to exert BK opening activity (Hu et al., *Drug.Dev.Res.* 41, 10 (1997)), benzimidazole derivatives with K_{ATP} and BK opening activity (US 5,475,015), novel compounds (eg. NS004) with K^+ channel opening activity by Neurosearch (WO 00/69838; WO 00/34248) and 3-substituted oxoindole derivatives with BK-channel opening activity for neuronal protection, especially after ischemic stroke (US 5,602,169).

In general, the present invention provides compounds useful for the treatment or alleviation of diseases, disorders, and conditions associated with potassium channels.

The present invention is therefore directed to compounds of the general Formula (I)



wherein

R is a monocyclic or polycyclic substituted or unsubstituted aromatic ring system which may contain one or more groups X and which contains at least one aromatic ring;

X is selected from the group consisting of S, O, N, NR\`, SO or SO_2;

Suitable substituents for R are halogen, CF_3, OCF_3, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR\`O, $CO_2R$\`, alkoxy, alkylthio, substituted or unsubstituted alkylaryl, alkylsulfonyl;

R` is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, hydroxyalkyl, haloalkyl, hydroxyalkylamine, amine, alkylamine, substituted or unsubstituted alkylaryl, aryl or heteroaryl.

The invention also provides a pharmaceutical composition comprising a compound of Formula (I), in free form or in the form of pharmaceutically acceptable salts or physiologically functional derivatives together with a pharmaceutically acceptable diluent or carrier therefore.

The term "physiologically functional derivative" as used herein refers to compounds which are not pharmaceutically active themselves but which are transformed into their pharmaceutically active form *in vivo*, i.e. in the subject to which the compound is administered. The physiologically functional derivative may be an ester, amide or sulfamide derivative of the compound of Formula (I) or of a salt thereof.

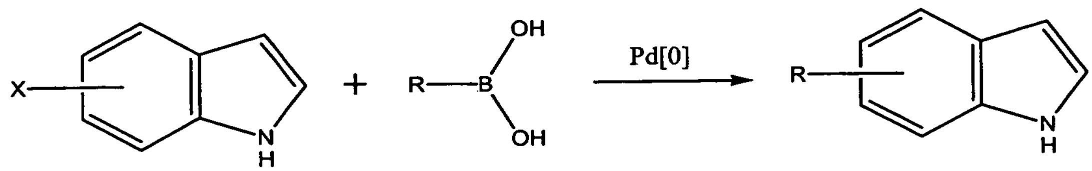
In another aspect, the present invention also provides a method for the treatment or prophylaxis of a condition where there is an advantage in regulating the membrane potential and/or conductance in cells of mammals, including a human, by the specific modulation of potassium channels which comprises the administration of an effective amount of a compound of Formula (I) and physiologically acceptable salts or physiologically functional derivatives thereof.

The invention is also directed to the use of compounds of the Formula (I) and of their pharmacologically tolerable salts or physiologically functional derivatives for the production of a medicament for the prevention, alleviation and/or treatment of diseases in mammals, including a human, responsive to the specific modulation of potassium channels.

In addition, the present invention provides methods for preparing the desired indole of the Formula (I).

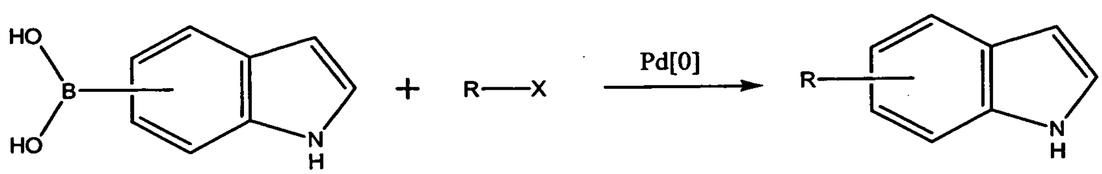
A first method for synthesis of the arylindole of the Formula (I) comprises the step of reacting an arylhalide [T.Oh-e, N. Miyaura, A. Suzuki, *J. Org. Chem.* (1993), *58*, 2201-

2208; W. A. Herrmann, V. P.W. Böhm, C.-P Reisinger, *J. Organomet. Chem.* (1999), *576*, 23-41; S. P. Stanforth, *Tetrahedron* (1998), *54*, 263-303; N. Miyaura, A. Suzuki, *Chem. Rev.* (1995), *95*, 2457-2483; A. Suzuki, *J. Organomet. Chem.* (1999), *576*, 147-168; A. Bahl, W. Grahn, S. Stadler, F. Feiner, G. Bourhill, C. Bräuchle, A. Reisner, P.G. Jones, *Angew. Chem. Int. Ed. Engl.* (1995), *34*, 1485-1488.] or aryltriflate of Formula (II) with an aryl boronic acid of the Formula (III) [T.Oh-e, N. Miyaura, A. Suzuki, *J. Org. Chem.* (1993), *58*, 2201-2208].



Formula II Formula III Formula I

A second method of the invention for preparing the compounds of Formula (I) comprises the step of reacting an indole boronic acid of the Formula (IV) with an arylhalide or aryltriflate of the general Formula (V).



Formula IV Formula V Formula I

In a preferred embodiment of the invention, R is an aromatic mono- or bicyclic hydrocarbon group having 5 to 15 carbon atoms, in particular having 5 to 10 carbon atoms, which optionally contains 1-4 N and/or O and/or S heteroatoms, in particular by 1 to 3 of these heteroatoms. Preferably, R is selected from a phenyl, furan, thiophene, oxazole, thiazole, isoxazole, isothiazole, 1,2,3-triazole, 1,3,4-thiadiazole, pyran, indole, isoindole, pyridine, pyridazine, pyrimidine, pyrazine, indazole, benzimidazole, triazine, indolizine, benzofuran, benzothiophene, benzothiophene-1,1-dioxide, benzothiazole, purine,

quinolizine, quinoline, isoquinoline, cinnoline, phthalazine, quinazoline, naphthyridine and pteridine group. Particularly preferred compounds are those in which R is a phenyl group or thiophene.

One or more of the carbon atoms in the ring system R can be substituted by a group X, wherein X is selected from the group consisting of S, O, N, NR`, SO or SO_2. In one preferred embodiment, one of the carbon atoms is substituted by a group X.

In other preferred embodiments, preferred optional substituents of R are F, CF_3, OCF_3, Cl, OCH_3, or C_1-C_5-alkyl, preferably F, CF_3, OCF_3.

In a preferred embodiment of the invention, R is a phenyl group in the 4- position to the indol group of the compound of the Formula (I) and one or more substituents of R are in ortho-, meta-, or para- position of the phenyl group and preferably represent halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, most preferably F, CF_3, OCF_3.

In another preferred embodiment of the invention, R is a phenyl group in the 5- position to the indol group of the compound of the Formula (I) and one or more substituents of R are in ortho-, meta-, or para- position of the phenyl group and the substituents of R are preferably halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, most preferably F, CF_3, OCF_3.

In another preferred embodiment of the invention, R is a phenyl group in the 6- position to the indol group of the compound of the Formula (I) and one or more substituents of R are in ortho-, meta-, or para- position of the phenyl group and the substituents of R are preferably halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, most preferably F, CF_3, OCF_3.

In another preferred embodiment of the invention, R is a phenyl group in the 7- position to the indol group of the compound of the Formula (I) and one or more substituents of R are in ortho-, meta-, or para- position of the phenyl group and the substituents of R are

preferably halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, most preferably F, CF_3, OCF_3.

In another preferred embodiment of the invention, R is 3-thienyl moiety in the 4- position to the indol group of the compound of the Formula (I) and one or more substituents of R are in 2- position of the thienyl moiety and the substituents of R are preferably halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, most preferably F, CF_3, OCF_3.

In another preferred embodiment of the invention, R is 3-thienyl moiety in the 5- position to the indol group of the compound of the Formula (I) and one or more substituents of R are in 2- position of the thienyl moiety and the substituents of R are preferably halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, most preferably F, CF_3, OCF_3.

In another preferred embodiment of the invention, R is 3-thienyl moiety in the 6- position to the indol group of the compound of the Formula (I) and one or more substituents of R are in 2- position of the thienyl moiety and the substituents of R are preferably halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, most preferably F, CF_3, OCF_3.

In another preferred embodiment of the invention, R is 3-thienyl moiety in the 7- position to the indol group of the compound of the Formula (I) and one or more substituents of R are in 2- position of the thienyl moiety and the substituents of R are preferably halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, most preferably F, CF_3, OCF_3.

The compounds of the Formula (I) to be used according to the invention can form salts with inorganic or organic acids or bases. Examples of such salts are, for example ammonium salts.

An alkyl group, if not stated otherwise, is preferably a linear or branched chain of 1 to 6 carbon atoms, preferably a methyl, ethyl, propyl, isopropyl, butyl, *t*-butyl, isobutyl, pentyl or hexyl group, a methyl, ethyl, isopropyl or *t*-butyl group being most preferred.
The alkyl group in the compounds of formula (I) can optionally be substituted by one or more substituents R`, preferably by halogen.

An alkylsulfonyl group denotes an (SO_2)-alkyl group, the alkyl group being defined above.

An cycloalkyl group denotes a non-armoatic ring system containing 4 to 8 carbon atoms, wherein the ring system comprises one or more of the carbon atoms in the ring can be substituted by a group X, X being as defined above.

An alkoxy group denotes an O-alkyl group, the alkyl group being as defined above.

An alkylthio group denotes an S-alkyl group, the alkyl group being as defined above.

An haloalkyl group denotes an alkyl group which is substituted by one to five preferably three halogen atoms, the alkyl group being as defined above.

A hydroxyalkyl group denotes an HO-alkyl group, the alkyl group being as defined above.

An haloalkyloxy group denotes an alkoxy group which is substituted by one to five preferably three halogen atoms, the alkyl group being as defined above.

A hydroxyalkylamino group denotes an $(HO\text{-alkyl})_2$-N- group or HO-alkyl-NH- group, the alkyl group being as defined above.

An alkylamino group denotes an HN-alkyl or N-dialkyl group, the alkyl group being as defined above.

An aminoalkyl group denotes an H_2N-alkyl, monoalkylaminoalkyl, or dialkylaminoalkyl group, the alkyl group being as defined above.

A halogen group is chlorine, bromine, fluorine or iodine, fluorine being preferred.

An aryl group preferably denotes an aromatic group having 5 to 15 carbon atoms, in particular a phenyl group. This aryl group can optionally be substituted by one or more substituents R`, where R` is as defined above, preferably by haloalkyloxy.

An arylalkyl group denotes an alky group which is substituted by one to three preferably one aryl groups, the alkyl and aryl group being as defined above.

A heteroaryl group denotes a 5- or 6-membered heterocyclic group which contains at least one heteroatom like O, N, S. This heterocyclic group can be fused to another ring. For example, this group can be selected from an oxazol-2-yl, oxazol-4-yl, oxazol-5-yl, thiazol-2-yl, thiazol-4-yl, thiazol-5-yl, isothiazol-3-yl, isothiazol-4-yl, isothiazol-5-yl, 1,2,4-oxadiazol-3-yl, 1,2,4-oxadiazol-5-yl, 1,2,4-thiadiazol-3-yl, 1,2,4-thiadiazol-5-yl, 1,2,5-oxadiazol-3-yl, 1,2,5-oxadiazol-4-yl, 1,2,5-thiadiazol-3-yl, 1-imidazolyl, 2-imidazolyl, 1,2,5-thiadiazol-4-yl, 4-imidazolyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 2-furanyl, 3-furanyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 3-pyridazinyl, 4-pyridazinyl, 2-pyrazinyl, 1-pyrazolyl, 3-pyrazolyl, 4-pyrazolyl, indolyl, indolinyl, benzo-[b]-furanyl, benzo[b]thiophenyl, benzimidazolyl, benzothiazolyl, quinazolinyl, quinoxazolinyl, or preferably isoxazol-3-yl, isoxazol-4-yl, isoxazol-5-yl, quinolinyl, tetrahydroquinolinyl, isoquinolinyl, tetrahydroisoquinolinyl group. This heterocyclic group can optionally be substituted by one or more substituents R`, where R` is as defined above.

In general, the compounds of the present invention will be useful in the treatment of disorders of a living animal body, including a human, due to their potent potassium channel modulating properties.

Therefore, the compounds of the instant invention will be useful in treating disorders of mammals, including humans, where the modulation of the membrane potential

or ion conductances is influencing the effects of the disorders. Such disorders include asthma, cystic fibrosis, obstructive pulmonary disease, convulsions, vascular spasms, urinary incontinence, urinary instability, urinary urgency, bladder spasms, ischemia, cerebral ischemia, traumatic brain injury, neurodegeneration, migraine, pain, psychosis, hypertension, epilepsy, memory and attention deficits, functional bowel disorders, erectile dysfunction, immune suppression, autoimmune disorders, dysfunction of cellular proliferation, diabetes, premature labour, and other disorders associated with or responsive to the modulation of potassium channels.

The invention provides a pharmaceutical formulation comprising a compound of Formula (I) of the invention or a pharmaceutically acceptable salt or derivative thereof, together with one or more pharmaceutically acceptable carriers therefore, and optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous, intradermal, and intraveneous) administration or in a form suitable for administration by inhalation or insufflation. The compounds of the invention, together with a conventional adjuvant, carrier, or diluent, may thus be placed into the form of pharmaceutical compositions and unit dosages thereof, and in such form may be employed as solids, liquids or in the form of sterile injectable solutions. If a solid carrier is used, the preparation may be tableted, placed in a hard gelatine capsule in powder or pellet form, or in form of a troche or lozenge. The solid carrier may contain conventional excipients such as binding agents, tableting lubricants, fillers, disintegrants, wetting agents and the like. Tablets may be film coated by conventional techniques. If a liquid carrier is employed, the preparation may be in form of a syrup, emulsion, soft gelatine capsule, sterile vehicle for injection, an aqueous or non-aqueous liquid suspension, or may be a dry product for reconstitution with water or other suitable vehicles before use. Liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, wetting agents, non-aqueous vehicle (including edible oils), preservatives, as well as flavouring and /or colouring agents. For parenteral administration, a vehicle normally will comprise sterile water, at least in large part, although saline solutions, glucose solutions and like may

be utilized. Injectable suspensions also may be used, in which case conventional suspending agents may be employed. Conventional preservatives, buffering agents and the like also may be added to the parenteral dosage forms. Administration, however, can also be carried out rectally, e.g., in the form of suppositories, or vaginally , e.g. in the form of pessaries, tampons, creams, or percutaneously, e.g., in the form of ointments, creams or tinctures. Administration directly to the nasal cavity by conventional means can be carried out e.g. by pipette, spray or dropper, administration to the respiratory tract may be achieved by means of an aerosol formulation, e.g. where the active ingredient is provided in a pressurized pack with a suitable propellant, or other suitable application mechanisms.

The pharmaceutical compositions are prepared by conventional techniques appropriate to the desired preparation containing appropriate amounts of the active ingredient, that are, the compounds in this invention. Such pharmaceutical compositions and unit dosage forms thereof may comprise conventional ingredients in conventional proportions, with or without additional active compounds or principles, and such unit dosage forms may contain any suitable effective amount of the active ingredient commensurate with the intended daily dosage range to be employed.

A suitable dose of compounds or pharmaceutical compositions thereof for a mammal, especially humans, suffering from, or likely to suffer from any condition as described herein is an amount of active ingredient from about 0.1μg/kg to 500mg/kg body weight. For parenteral administration, the dose may be in the range of 0.1μg/kg to 100mg/kg body weight for intravenous administration. The active ingredient will preferably be administered in equal doses from one to four times daily. The compounds of Formula (I) can also be used in the form of a precursor (prodrug) or a suitably modified form that releases the active compound *in vivo*. Normally, the administered dose will be gradually increased until the optimal effective dosage for the treated host is determined. The optimal administered dosage will be determined by a physician or others skilled in the art, depending on the relevant circumstances including the condition to be treated, the choice of compound to be administered, the route of administration, the sex, age, weight, and the specific response of the treated individual in respect to the severity of the individual's symptoms.

Examples

1. Synthesis of compounds of Formula (I)

First method of synthesis:

4-, 5-, 6- or 7-haloindole, or 4-,5-,6- or 7-triflateindole (100 mg, 1eq), aryl- or heteroaryl-boronic acid (1.2 eq), $Pd(PPh_3)_4$ (0.03 eq), and barium hydroxide octahydrate (3.3 eq) were dissolved in a mixture of toluene (2 ml) - ethanol (2 ml) - water (1 ml). The mixture was stirred for 16 h at 100 °C and then cooled to room temperature [TLC (*n*-hexane-EtOAc, 8:2)]. The crude product was purified either directly by preparative thin layer chromatography (Merck, 20 x 20 cm, Silica gel 60 F_{254}, 1 mm) using (*n*-hexane:EtOAc, 9:1) as eluent, or filtered through a pad of celite, concentrated, and then purified by preparative thin layer chromatography [A. Suzuki, *J. Organomet. Chem.* (1999), *576*, 147-168; A. Bahl, W. Grahn, S. Stadler, F. Feiner, G. Bourhill, C. Bräuchle, A. Reisner, P.G. Jones, *Angew. Chem. Int. Ed. Engl.* (1995), *34*, 1485-1488].

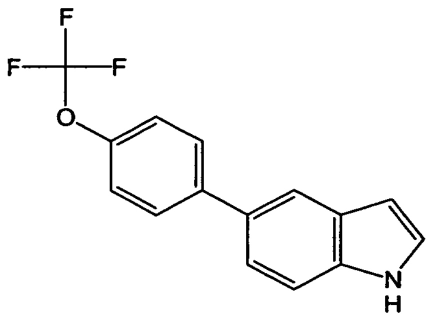
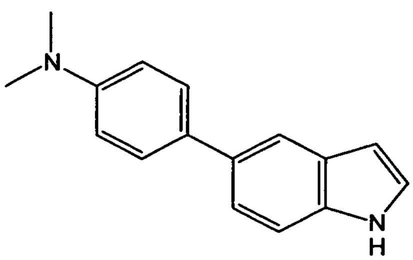
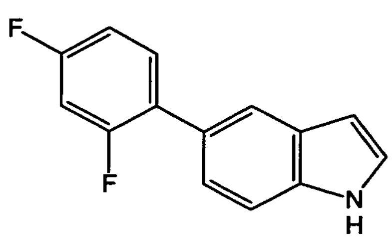
Second method of synthesis:

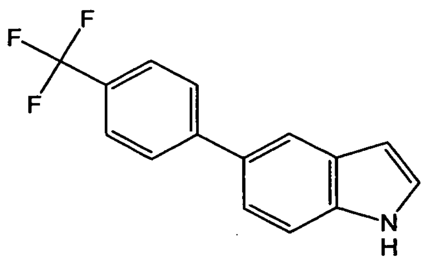
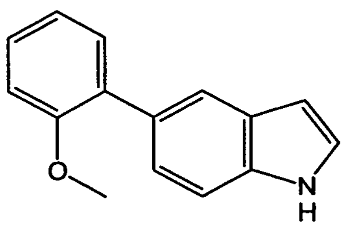
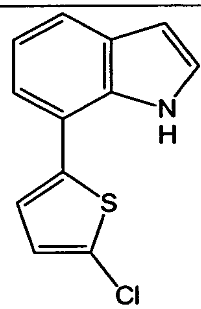
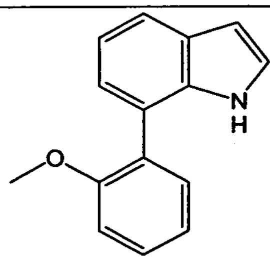
4-, 5-, 6- or 7-indole boronic acid (1,2 eq), haloaryl or heterohaloaryl or aryltriflate (100mg, 1 eq), $Pd(PPh_3)_4$ (0.03 eq), and barium hydroxide octahydrate (3.3 eq) were dissolved in a mixture of toluene (2 ml) - ethanol (2 ml) - water (1 ml). The mixture was stirred for 16 h at 100 °C and then cooled to room temperature [TLC (*n*-hexane-EtOAc, 8:2)]. The crude product was purified either directly by preparative thin layer chromatography (Merck, 20 x 20 cm, Silica gel 60 F_{254}, 1 mm) using (*n*-hexane:EtOAc, 9:1) as eluent, or filtered through a pad of celite, concentrated, and then purified by preparative thin layer chromatography.

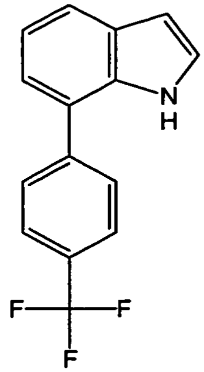
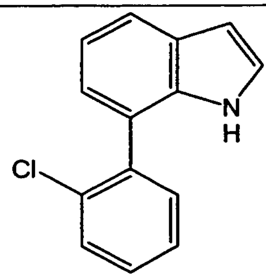
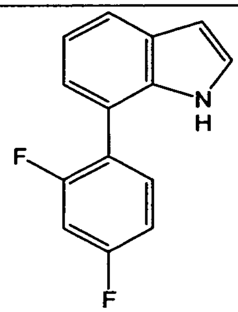
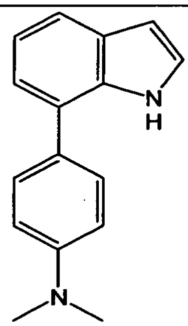
[A. Suzuki, *J. Organomet. Chem.* (1999), *576*, 147-168; A. Bahl, W. Grahn, S. Stadler, F. Feiner, G. Bourhill, C. Bräuchle, A. Reisner, P.G. Jones, *Angew. Chem. Int. Ed. Engl.* (1995), *34*, 1485-1488].

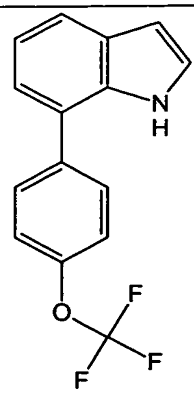
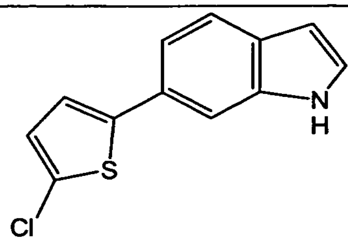
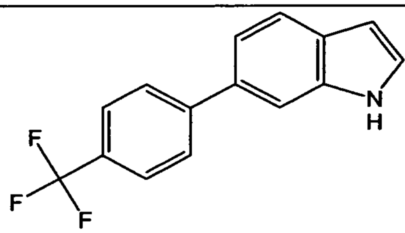
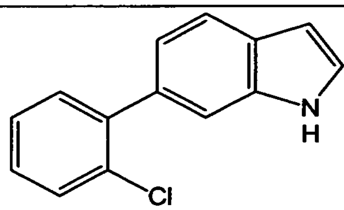
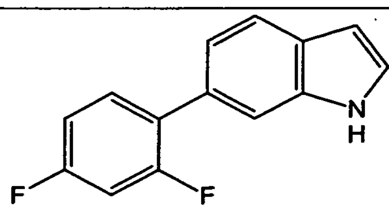
Table I: Mass was determined by mass spectrometry, the exact molecular mass, the NMR data (abbreviations: br. = broad, s = singulet, d = doublet, t = triplet, m = multiplet, J = ^{1}H-^{1}H coupling constant) and the E_m assay results are shown. E_m Assay results are given as the ratio of the compound effect (50µM) compared to the maximal effect of NS004 (25 or

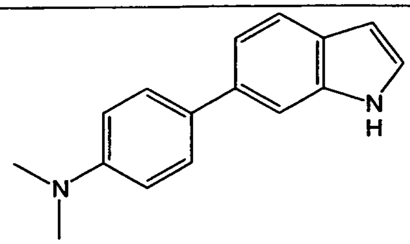
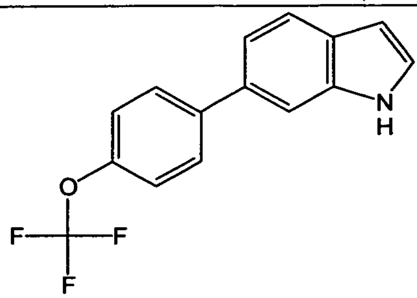
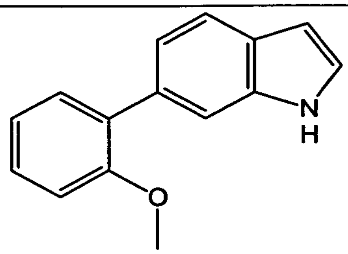
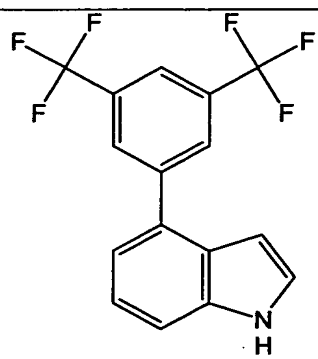
50μM). Ranges are 0-1 = +, >1= ++, blocking effects (showing increased fluorescence intensity) are given as -.

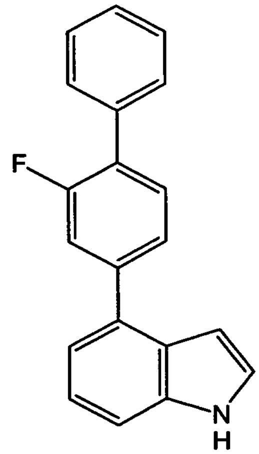
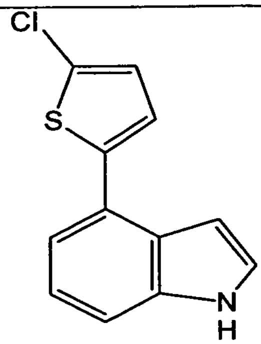
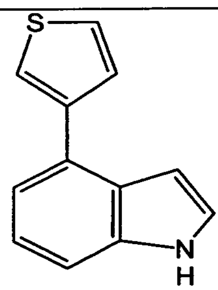
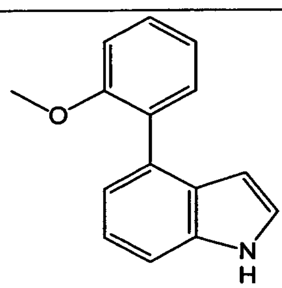
N	Structure	HPLC/ MS (ESI)	^{1}H-NMR (300 MHz)	E_m effect
1		278 $[M+H]^+$ 276 $[M-H]^+$	δ ($CDCl_3$) = 6.52 (s, 1 H, H-3), 7.14-7.20 (m, 3 H, H-2, H-3' and H-5'), 7.29-7.37 (m, 2 H, H-6 and H-7), 7.54 (d, J = 8.4 Hz, 2 H, H-2' and H-6'), 7.73 (s, 1 H, H-4), 8.04 (s, 1 H, NH)	++
2		237 $[M+H]^+$	δ ($CDCl_3$) = 2.92 (s, 6 H, $N(CH_3)_2$), 6.50 (br. s, 1 H, H-3), 6.85 (d, J = 7.3 Hz, 2 H, H-3' and H-5'), 7.14 (br. s, 1 H, H-2), 7.34 (s, 2 H, H-6 and H-7), 7.49 (d, J = 8.4 Hz, 2 H, H-2' and H-6'), 7.72 (s, 1 H, H-4), 8.08 (s, 1 H, NH)	+
3		230 $[M+H]^+$ 228 $[M-H]^+$	δ ($CDCl_3$) = 6.51 (br. s, 1 H, H-3), 6.79-6,89 (m, 2 H, H-3' and H-5'), 7.16 (br. s, 1 H, H-2), 7.25 (d, J = 8.4 Hz, 1 H, H-6'), 7.35-7.39 (m, 2 H, H-6 and H-7), 7.68 (s, 1 H, H-4), 8.10 (s, 1 H, NH)	++

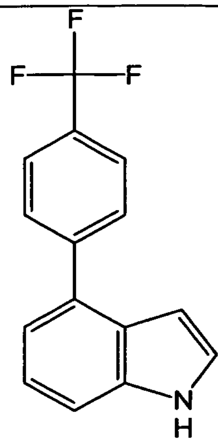
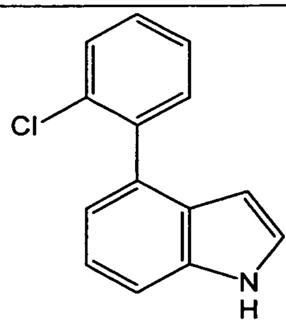
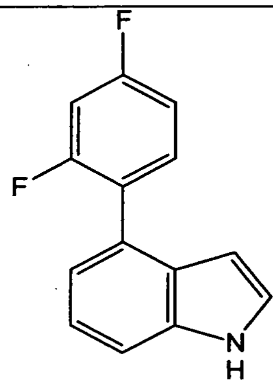
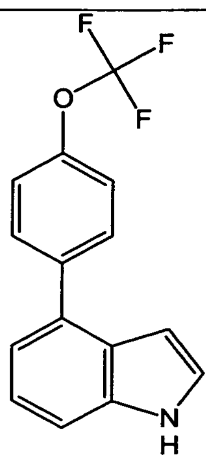
4		262 [M+H]$^+$ 260 [M-H]$^+$	δ (CDCl$_3$) = 6.54 (br. s, 1 H, H-3), 7.18 (br. s, 1 H, H-2), 7.38 (s, 2 H, H-6 and H-7), 7.59 (d, J = 8.7 Hz, 2 H, H-2' and H-6'), 7.66 (d, J = 8.3 Hz, 2 H, H-3' and H-5'), 7.79 (s, 1 H, H-4), 8.12 (s, 1 H, NH)	++
5		224 [M+H]$^+$	δ (CDCl$_3$) = 3.71 (s, 3 H, OCH$_3$), 6.89-6,97 (m, 2 H, H-3' and H-5'), 7.07 (br. s, 1 H, H-2), 7.20 (t, J = 6.9 Hz, 1 H, H-4'), 7.29-7.31 (m, 3 H, H-6, H-7 and H-6'), 7.69 (s, 1 H, H-4), 7.99 (s, 1 H, NH)	++
6		234 [M+H]$^+$ 232 [M-H]$^+$		+
7		224 [M+H]$^+$		+

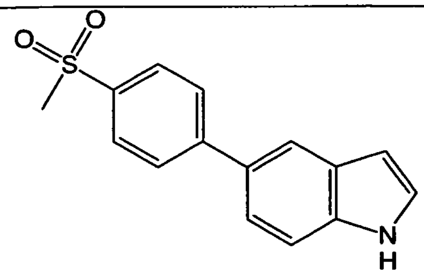
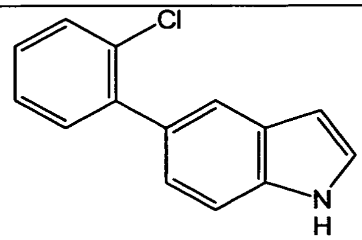
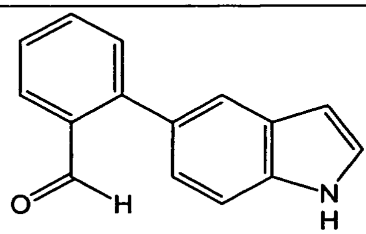
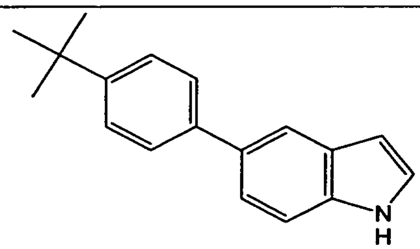
8		262 [M+H]+ 260 [M-H]+		+
9		228 [M+H]+		+
10		230 [M+H]+ 228 [M-H]+		+
11		237 [M+H]+		++

12		278 [M+H]$^+$ 276 [M-H]$^+$		-
13		234 [M+H]$^+$ 232 [M-H]$^+$		+
14		262 [M+H]$^+$ 260 [M-H]$^+$		+
15		228 [M+H]$^+$		+
16		230 [M+H]$^+$ 228 [M-H]$^+$		+

17		237 [M+H]$^+$		++
18		278 [M+H]$^+$ 276 [M-H]$^+$		-
19		224 [M+H]$^+$		+
20		330 [M+H]$^+$ 328 [M-H]$^+$		+

21		288 [M+H]+ 286 [M-H]+		+
22		234 [M+H]+ 232 [M-H]+		+
23		200 [M+H]+ 198 [M-H]+		+
24		224 [M+H]+		+

25		262 [M+H]+ 260 [M-H]+		+
26		228 [M+H]+ 226 [M-H]+		+
27		230 [M+H]+ 228 [M-H]+		+
28		278 [M+H]+ 276 [M-H]+		+

29		272 $[M+H]^+$ 270 $[M-H]^+$		+
30		228 $[M-H]^+$		++
31		222 $[M+H]^+$ 220 $[M-H]^+$		+
32		250 $[M+H]^+$ 248 $[M-H]^+$		+

Biological activity

The large conductance, voltage-dependent and Ca^{2+}-activated potassium channel BK is a potassium selective ion channel and belongs to the subfamily of K_{Ca} channels. Four BK alpha-subunits form a functional channel that can be regulated by intracellular Ca^{2+} concentration, membrane voltage, and other mechanisms like phosphorylation states or beta subunits. To test the biological activity of the compounds, we applied two different techniques, a fluorescence based assay using a voltage sensitive dye (E_m-Assay) as well as exploiting electrophysiological methods.

E_m-Assay:

CHO cells permanently transfected with cloned *hSlo* (*α-hSlo* and *β-bSlo*), yielding typical BK potassium currents (Zhou *et al.*, *Pflügers Arch.*, 436: 725-734 (1998), were used for the evaluation of compound activity. Activation or inhibition of BK channels in these cells leads to a change of the electrochemical gradient causing a hyperpolarized or depolarised membrane potential, respectively.

To determine changes in the membrane potential of the cells we used the voltage sensitive dye $DiBAC_{(4)}3$ (Molecular Probes) in a kinetic assay system using a fluorescent plate reader (Manning and Sontheimer, *J. Neurosci. Meth.*, 91: 73-81 (1999). The anionic bis-oxonol $DiBAC_{(4)}3$ is a voltage sensitive dye which partitions from the extracellular environment into the cell where it reversibly binds to intracellular proteins, a kinetic process depending on the membrane potential of the cell. At depolarised potentials (i.e. at a reduced K^+ efflux due to blocked K^+ channels) the dye accumulates in the cell leading to an increased fluorescence intensity, due to its increased fluorescence if bound to cellular proteins. At hyperpolarized potentials (i.e. at an increased K^+ efflux due to the opening of K^+ channels), the dye partitions out of the cell causing a decreased fluorescence intensity.

hSlo transfected CHO cells where maintained in DMEM supplemented with 10% FCS, 250µg/ml Geneticin, 100µg/ml Hygromycin, 1xHT-Supplement, and 1xNon-essential Amino Acids and cultured in a humidified CO_2 incubator. After trypsination, cells where plated with a density of $5x10^4$ cells per well on a clear 96-well plate and incubated for 24h. Cells where washed once with PBS, once with PBS containing 20mM HEPES (adjusted to pH 7.4 with NaOH) and 2µM $DiBAC_{(4)}3$ (DPBS-DiBAC solution). 180µl of the dPBS-DiBAC solution was then added to the cells and the plate incubated for 30-60 min at 37°C. During this time the dye could partition into the cells and reach a certain steady-state distribution, depending on the resting membrane potential. Test and reference compounds were stored as DMSO stock solutions and diluted in dPBS-DiBAC solution to the desired concentration.

Fluorescence intensity (Ex.: 485nm/Em.: 520nm) of each well was detected in the plate reader (Fluostar, BMG) every 60 seconds. After recording the baseline fluorescence for 7 minutes, 20µl test- and reference compounds were added and the fluorescence intensity was detected for additional 15 minutes. Background was subtracted, data values were normalized and expressed as a change in fluorescence intensity against time. The

change in fluorescence intensity caused by the test compounds was evaluated, compared to the effect of the reference compound NS004, and the ratio was determined (see Table I).

Electrophysiological studies:

CHO cells permanently transfected with cloned *α-hSlo* and *β-bSlo* were maintained as described above and used for electrophysiological characterisation. The whole-cell configuration of the patch-clamp technique was used to determine the effect of modulators on BK currents in these cells. The cell line expressing functional BK currents (Zhou et al., *Pflügers Arch.* 436, p.725 (1998)) were plated onto glass cover slips with a density of 1-$5x10^4$ cells/cover slip, incubated (37°C, 5% CO_2) and used for patch-clamp experiments within 24-48 h. Cells were bathed in mammalian ringer solutions containing (in mM): 160NaCl, 4.5KCl, $2CaCl_2$, $1MgCl_2$, 10HEPES, adjusted to pH 7.4, 290-310 mOsm. The internal pipette solution contained (in mM): 160KCl, $2CaCl_2$, $1MgCl_2$, 10 HEPES, EGTA was added to reach a free $[Ca^{2+}]_{internal}$ = $1x10^{-6}$M, adjusted to pH 7.2, 290-310 mOsm. Borosilicate pipettes with a resistance of 2-3 MΩ were filled with the internal solution and mounted on an appropriate holder. Prior to measurements a recording chamber was mounted onto the cell-plated cover slips and the cells were perfused with a simple syringe driven perfusion system. Compounds were added in the final concentration ($2x10^5$M) to the bath solution using the same system. An EPC-9 patch-clamp amplifier with Pulse and PulseFit software (HEKA) was used to record and analyze currents.

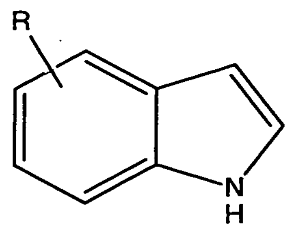
After addition of the compounds to the bath solution their modulating effect was determined by the increase or decrease of specific BK currents after reaching steady-state relative to the BK current before application of drugs (see Table II).

Table II: Results from the electrophysiological studies are given as the ratio of current increase after application of compound (20μM) relative to the control current before compound application. Currents were determined after reaching steady-sate. Ranges are 1 – 1.1 = +, >1,1 – 1.2 = ++, >1.2 = +++

Compound #	Mass	Effect
1	277	++
2	236	+
3	229	+++
4	261	++
5	223	++
22	233	++
30	227	+

Patent claims

1. The use of a compound of formula (I)



or a salt, a physiologically functional derivative, or a prodrug thereof, wherein

R is a monocyclic or polycyclic substituted or unsubstituted aromatic ring system which may contain one or more groups X and which contains at least one aromatic ring;

X is selected from the group consisting of S, O, N, NR`, SO or SO_2;

wherein substituents for R are halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, substituted or unsubstituted alkylaryl, alkylsulfonyl;

R` is hydrogen, alkyl, cycloalkyl, hydroxyalkyl, haloalkyl, hydroxyalkylamine, amine, alkylamine, substituted or unsubstituted alkylaryl, aryl or heteroaryl;

wherein an alkyl group and the alkyl parts of the above groups denote a linear or branched chain of 1 to 6 carbon atoms which is optionally substituted by one or more substituents R`, wherein R` is as defined above;

a cycloalkyl group denotes a non-aromatic ring system containing 4 to 8 carbon atoms, wherein one or more of the carbon atoms in the ring is optionally substituted by a group X, X being as defined above;

an alkylsulfonyl group denotes an (SO_2)-alkyl group;

an alkoxy group denotes an O-alkyl group;

an alkylthio group denotes an S-alkyl group;

a haloalkyl group denotes an alkyl group which is substituted by one to five halogen atoms;

a hydroxyalkyl group denotes an HO-alkyl group;

a haloalkyloxy group denotes an alkoxy group which is substituted by one to five halogen atoms;

a hydroxyalkylamino group denotes an $(HO\text{-alkyl})_2$-N- group or HO-alkyl-NH- group;

an alkylamino group denotes an NH-alkyl or N-dialkyl group;

an aminoalkyl group denotes an NH_2-alkyl, monoalkylaminoalkyl, or dialkylaminoalkyl group;

an aryl group preferably denotes an aromatic group having 5 to 15 carbon atoms which is optionally substituted by one or more substituents R\`, wherein R\` is as defined above;

an arylalkyl group denotes an alkyl group which is substituted by one to three aryl groups as defined above;

a heteroaryl group denotes a 5- or 6-membered heterocyclic group which contains at least one heteroatom O, N, or S, which is optionally fused to another ring, and which is optionally substituted by one or more substituents R\`, wherein R\` is as defined above;

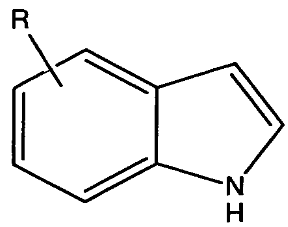
for the preparation of a medicament for the modulation of potassium channels.

2. The use according to Claim 1 wherein the medicament is used for the prevention, alleviation or treatment of diseases, conditions or disorders which are associated with, or dependent on the membrane potential or conductance of cells in mammals, including a human.

3. The use according to Claim 1 or 2 wherein the diseases are asthma, cystic fibrosis, obstructive pulmonary disease, convulsions, vascular spasms, urinary incontinence, urinary instability, urinary urgency, bladder spasms, ischemia, cerebral ischemia, traumatic brain injury, neurodegeneration, migraine, pain, psychosis, hypertension, epilepsy, memory and attention deficits, functional bowel disorders, erectile dysfunction, immune suppression, autoimmune disorders, dysfunction of cellular proliferation, diabetes, premature labour, or other disorders associated with or responsive to the modulation of potassium channels.

Abstract

The invention relates to compounds having the formula (I)



or a salt, a physiologically functional derivative, or a prodrug thereof, wherein

R is a monocyclic or polycyclic substituted or unsubstituted aromatic ring system which may contain one or more groups X and which contains at least one aromatic ring;

X is selected from the group consisting of S, O, N, NR`, SO or SO_2;

wherein substituents for R are halogen, CF_3, OCF_3, alkyl, cycloalkyl, haloalkyl, haloalkyloxy, hydroxyalkyl, hydroxyalkylamine, amine, aminoalkyl, alkylamine, CR`O, $CO_2$R`, alkoxy, alkylthio, substituted or unsubstituted alkylaryl, alkylsulfonyl;

R` is hydrogen, alkyl, cycloalkyl, hydroxyalkyl, haloalkyl, hydroxyalkylamine, amine, alkylamine, substituted or unsubstituted alkylaryl, aryl or heteroaryl;

wherein an alkyl group and the alkyl parts of the above groups denote a linear or branched chain of 1 to 6 carbon atoms which is optionally substituted by one or more substituents R`, wherein R` is as defined above;

a cycloalkyl group denotes a non-aromatic ring system containing 4 to 8 carbon atoms, wherein one or more of the carbon atoms in the ring is optionally substituted by a group X, X being as defined above;

an alkylsulfonyl group denotes an (SO_2)-alkyl group;

an alkoxy group denotes an O-alkyl group;

an alkylthio group denotes an S-alkyl group;

a haloalkyl group denotes an alkyl group which is substituted by one to five halogen atoms;

a hydroxyalkyl group denotes an HO-alkyl group;

a haloalkyloxy group denotes an alkoxy group which is substituted by one to five halogen atoms;

a hydroxyalkylamino group denotes an $(HO\text{-alkyl})_2$-N- group or HO-alkyl-NH- group;

an alkylamino group denotes an NH-alkyl or N-dialkyl group;

an aminoalkyl group denotes an NH_2-alkyl, monoalkylaminoalkyl, or dialkylaminoalkyl group;

an aryl group preferably denotes an aromatic group having 5 to 15 carbon atoms which is optionally substituted by one or more substituents R`, where R` is as defined above;

an arylalkyl group denotes an alkyl group which is substituted by one to three aryl groups as defined above;

a heteroaryl group denotes a 5- or 6-membered heterocyclic group which contains at least one heteroatom O, N, or S, which is optionally fused to another ring, and which is optionally substituted by one or more substituents R`, wherein R` is as defined above;

for the preparation of a medicament for the modulation of potassium channels.